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Effects of predator satiation on seed predation in new roadside prairie plantings

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EFFECTS OF PREDATOR SATIATION ON SEED PREDATION IN NEW ROADSIDE PRAIRIE PLANTINGS

An Abstract of a Thesis

Submitted

in Partial Fulfillment

Of the Requirements for the Degree

Master of Science

Jessica Riebkes

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ABSTRACT

Restoration efforts in the tallgrass prairie ecosystem are inhibited by high seed cost and as little as 10% emergence of planted, pure live seed. This study examined the portion of loss due to seed predation and sought to reduce this predation in new roadside prairie plantings. Studies document the occurrence of predation in several plant communities and across all plant families, but little is known about how to reduce the impact of seed predators, especially in a restoration setting. On three sites where native prairie seed was recently drilled, we attempted to satiate seed predators by broadcasting a supplemental food source—birdseed at ten times the rate of the prairie seed. The goal of this method was to capitalize on the evolutionary principals of optimal diet theory and masting in order to protect seed from predation. We quantified seed predation through the use of a buffet experiment during the same fall as the planting, and by monitoring early seedling establishment the following summer. We predicted a reduced loss of prairie seed in the supplemental seed treatment of the buffet experiment. During the growing season, we expected to find increased seedling establishment in the supplemental seed treatment. Results of the buffet experiment show limited seed predation, with no significant effect of the supplemental seed treatment and temporal variation at each of the sites. As this data was collected after a frost, lack of invertebrate seed predators could have influenced the low rates of predation. Results from the growing season showed that the supplemental seed treatment increased early seedling establishment, yielding 37% more seedlings than in

control plots. Detecting a treatment effect in the summer, but not in the fall may suggest that these plantings did not face high predation pressure this late in the fall, but that predators found the seeds sometime over the winter or in the spring. Further studies should test the most optimal time of year to apply the treatment and look at the effect of the treatment on sites with different disturbance characteristics. It is also important to use the treatment along with the recommended management guidelines for the site. Reducing predation on prairie seed through the use of supplemental seed could provide a practical, inexpensive strategy to improve prairie restorations across the Midwest.

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This Study by: Jessica Riebkes

Entitled: Effects of Predator Satiation on Seed Predation in New Roadside Prairie Plantings

has been approved as meeting the thesis requirement for the

Degree of Master of Science

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CHAPTER 1

INTRODUCTION

Grasslands, wetlands, forests, and other ecosystems across the globe have been exploited by humans. In the Upper Midwestern United States, where the development of land for agriculture is widespread, the area of native tallgrass prairie has declined by as much as 99.9% (Samson and Knopf 1994; Smith 1998). Restoration projects are occurring in order to reestablish native communities and the services they once provided. However, each ecosystem presents its own set of challenges to overcome. Tallgrass prairie restoration efforts are primarily inhibited by disappointing attempts at seed addition (Sluis 2002). This may be partly due to high seed cost and very low emergence of planted, pure live seed. It is common practice to sow 400 to 950 pure, live seeds (PLS) per m^2 to achieve around 30 plants per m^2 in the final population, which is a 3.1% to 7.5% establishment rate (Smith et al. 2010). Commercial seed is available for native tallgrass prairie species, but seed costs are high. Low diversity plantings, including 20-30 basic species can cost around \$500-1500 ha^{-1}, while a high diversity mixture of 50-70 species can cost \$2600-5000 ha^{-1} (Prairie Moon Nursery 2011). If only a fraction of the seed germinates and becomes established plants, this begs the question of what happens to the other portion of seed.

Low rates of establishment can be explained by several seedling fates: germination, persisting as dormant seeds in the soil, or mortality. Mortality is the most common fate in this system, comprising seedling death, fungal disease, and

consumption of seeds by herbivores (Clark and Wilson 2003; Orrock et al. 2006). One study suggests that as long as a seed has not been buried or otherwise physically protected from predators, it is subject to consumption. However, even buried seeds may not be safe from pathogens and larger predators like birds and mammals (Chambers and MacMahon 1994). Seed predation is a ubiquitous phenomenon, affecting all plant families in all terrestrial and freshwater habitats (Janzen 1971).

Effects of Predation

The relative influence of seed predation versus other causes of mortality are poorly understood in prairie systems but it is known that predation is highly variable by species, location, and time of year. Many studies have begun to consider seed predation as a factor in recruitment, contributing to variable plant community composition (Heithaus 1981; Howe and Brown 1999). At the most basic, predation contributes to less seed available for germination, though this can occur at different degrees. In the mesic deciduous forests of West Virginia, seed predation by rodents destroyed up to 47% of *Jeffersonia diphylla* (L.) Pers. seeds and 24% of *Asarum canadense* L. seeds. When ants were excluded, rodent seed predators consumed 70% of *A. canadense* seeds (Heithaus 1981). A study conducted in a 30 year old restored tallgrass prairie in Iowa sought to quantify seed predation using a buffet experiment. In the fall, a known number of seeds were glued to cards and placed in the field. After set intervals of time, the number of undamaged seeds remaining on the cards were recorded. Across all species, 66% of seeds were removed from a site in the first 20 days and 84% were removed by the end

of the trial (58 days). Only 25% of *Silphium integrifolium* (Michx.) seeds were preyed upon in the first 20 days, but 96.3% of *Phlox pilosa* (L.) seeds and 84.4% of *Dodecatheon meadia* (L.) seeds were removed by day 20 (Hemsath 2007a). It is important to note that the nature of the experiment (i.e. gluing seeds to cards) may make the seeds more obvious, leading to exaggerated predation rates (Westerman et al. 2006; Baraibar et al. 2009). However, this is an acknowledged limitation of the buffet experiments.

Another Iowa study in the tallgrass prairie ecosystem further illustrates the variability and effects of seed predation. This study sought to identify whether the exclusion of vertebrate predators from three new prairie plantings would increase the number of seedlings that established. Within three hours of the summer prairie planting, wire exclosures were placed in the field. These exclosures had one closed side and one side that remained open, allowing vertebrates to enter. At several times during the growing season emerging seedlings were identified and counted. Results differed across the three sites, but overall there were more seedlings in the closed exclosures. The loss of seed to predation reduced seedling establishment by 9.8 seedlings/m^2 or 41% (Pellish 2014). While this methodology would not be practical for large scale projects, it demonstrates that protecting seeds from predation is a worthwhile and potentially cost saving measure for restoration projects.

There also appears to be variability in the types of predators that consume seeds. A study in another tallgrass community used exclosures to look at the effects of bird versus mammal predators. Eighteen plant species were selected and seed mixes

were broadcast seeded in the early summer at both a high density rate and a low density rate. Four-seven by seven meter exclosures were placed within experimental plots, one to exclude all vertebrates, one to admit birds and small rodents, one to exclude birds but admit rodents, and one to admit birds but exclude rodents. Seedlings were sampled in early October of the same year. Observational data revealed more than seven bird species visiting the plots, and live trapping yielded 5 different species of small mammals visiting the plots. Results showed that seed eating birds reduced grass biomass by 23% and rodents reduced forb biomass by up to 57%. Bird predators had a more dramatic effect on seedling numbers in the high density plantings than low density plantings (Howe and Brown 1999). Another study by Howe and Brown (2000) used exclosures at different times of the year to exclude rodents. Their work showed that meadow voles (*Microtus pennsylvanicus)* were highly selective seed predators on *Silphium integrifolium* during the winter, reducing the density of this species by 59%. This selective predation did not simply reduce *S. integrifolium* density, but may have caused compensatory increases in other species, further altering the community composition of the site. Suppression of this species allowed for increases in small-seeded species not eaten by voles by 24-132% (Howe and Brown 2000).

Since only a small fraction of planted seeds recruit to the seedling stage, seed establishment can play a big role in the plants found in a restoration (Clark et al. 2007). In addition, this loss of seeds is disproportionate to the loss in seedlings. On a study in native sand hills prairie, a three-fold reduction in seeds by insects led to a six-fold

decrease in seedling establishment (Louda, Potvin, and Collinge 1990). Seed predators can even affect plant composition as they consume some species preferentially (Howe and Brown 2000). It has been hypothesized that this differential granivory is at least partially due to seed size because in some cases, the largest seeded species suffered greater seed loss than other species. Small seeded species may even increase in abundance due to the predation on large seeded species (Howe and Brown 2000). In another study, exclusion of predators actually increased the number of seedlings of large seeded species, but not small seeded species. The authors speculated that the large seeded species normally faced higher predation pressure than small seeded species, so exclusion released this pressure and allowed large species to increase (Reader 1993). Levey et al. suggests that nutrition also comes into play, as some species prefer foods that are high in carbohydrates and low in lipids, whereas others prefer fruits low in carbohydrates and high in lipids (Levey et al. 2006).

Differential granivory may be due to which predators are present in a given ecosystem, season of the year, or at a certain temperature. For example, some weed seed studies show that rodents are the biggest predator in the fall and winter, but other studies saw low or insignificant rodent predation at that time of year (Westerman et al. 2008; Baraibar et al. 2009; Cardina et al. 2011). To the best of my knowledge, seasonality and predation has not been studied in tallgrass prairies, but it is likely that these patterns are different in land that has vegetative cover year round versus an agricultural system. Amount of vegetative cover or litter may influence seed predation

on its own, sometimes increasing predation, other times decreasing it (Baraibar et al. 2009; Reed, Kaufman, and Kaufman 2006; Westerman et al. 2006).

Another factor influencing seed predation is the density of seeds available. In some cases, seed removal increased with increased density of seed planted, but studies of weed science have shown less predation with increased density of seed (Ostfeld, Manson, and Canham 1997; Westerman et al. 2003; Cardina et al. 2011; Crawley and Edwards 2011). In these studies, high seed density may have overwhelmed the ability of consumers to limit or change plant populations. Similarly, a study in California grasslands indicated that doubling the planted seed density had no effect in plots where rodents were allowed access (Orrock, Witter, and Reichman 2009). In Iowa, predators did not increase or alter foraging patterns when seeds were added (Orrock et al. 2006). In some cases, interactions between the consumers and seed density of the planting are also possible. For example, a study on a tallgrass prairie community showed that bird effects were more pronounced in high-density plantings, while rodent effects were most pronounced in low-density plantings (Howe and Brown 1999). Little is known about the prairie system when it comes to how the seeding rate influences the amount of predation, though it seems possible that this could influence the outcome of the restoration. Knowing whether predation pressure is greater at a high seeding rate, or if predators are satiated by a high seeding rate would allow restoration practitioners to better design seed mixes.

While it is known that predation is a big force and that there are a suite of predators involved, less is known about how to reduce predation. This problem has drawn the attention of weed science researchers, who have shown that as many as 32-70% of weed seeds are consumed by predators—a significant ecosystem service for the farmer (Westerman et al. 2003). As predation of weed seeds is a beneficial to agronomists, researchers have begun to develop methods to test whether predation can be manipulated.

Capitalizing on the array of existing strategies that plants use against seed predators may be our best chance at manipulating predation. The attack on seeds by consumers is not a one-sided fight; plants deploy several different classes of defense against seed predators (Janzen 1971). Some of these strategies are physical mechanisms, like spikes, burrs, size, weight, stickiness, and shape. Others are chemical defenses, such as the secondary compounds of caffeine and capsaicin. While capsaicin may be a natural plant defense, experimental use of the compound has seen variable results. In one case, capsaicin had no effect for some species, but increased seedling recruitment of the prairie forb, Shooting Star (*Dodecatheon meadia* L.) (Hemsath 2007a). Another study showed that capsaicin could deter seed predation by small mammals, but allow seed dispersal by birds. The authors speculated that this compound may provide a mechanism by which plants can prevent consumption by mammalian seed predators without decreasing ingestion by avian seed dispersers (Levey et al. 2006). Along the same lines, artificial compounds have also been tested for their

capability to deter predators. Thiram, one such compound, is actually a fungicide marketed as Gustafson 42-SR2 and emits sulfurous odors, which repel predators. It was found to be effective on its own at repelling birds and deer mice, and in a mixture with capsaicin (Nolte and Barnett 2000; Ngowo et al. 2005). While using chemical deterrents such as plant secondary compounds to reduce predation holds promise, the strategy presents challenges as seed coatings are quickly and easily removed by rain, snow, wind, or other weathering. Waterproof coatings exist, like those used to apply herbicides/fungicides to agricultural seed, but are often patented formulas. Even with a good coating, applying it to a diverse mix of prairie seed would be challenging as all of the seeds are different sizes, shapes, and textures.

Spatial or temporal defenses put up by plants are other plant defenses that could be utilized. These defenses also hold much promise, as they too derive from key evolutionary principles. One of these principles is masting, a plant's ability to defend its seed by producing large, synchronized intermittent seed crops (Kelly 1994; Kelly et al. 2000; Kelly and Sork 2002). Janzen (1971) suggested that seed predators cause selection for masting when these large seed crops experience less seed predation than years with smaller seed crops. In high seed years, the seed crops would satiate the predators, resulting in lower overall seed loss. Furthermore, the principle of optimal diet theory predicts that predators will maximize their net energy intake per unit handling time, dropping lower value prey from the diet as the abundance of higher value prey increases (Janzen 1971; Sih and Christensen 2001). For example, ants offered millet in a

buffet experiment only carried off seeds with little to no husk (the smooth husk makes the seed harder to carry). Smooth seeds were still found in the ant's diet, as long as they were attached to the seed head, making it easier to transport. This suggests that seed shape prevented ants from taking certain seeds that would have increased the net time and difficulty of handling (Pulliam and Brand 1975). Similarly, Erasmus and Kerley (2011) showed that there was a correlation between the energy content of seeds and the rate of intake by rodents. As it is important for rodents to evaluate the cost of extracting seed compared to its caloric content, these principles may be a way to manipulate predation in a restoration setting.

Because of low seedling emergence rates in tallgrass prairie plantings, even marginal reductions in predation by these strategies could result in many-fold increases in seedling emergence. This study seeks to examine the portion of loss due to seed predation in new roadside prairie plantings and implement the principles of optimal diet theory and masting in order to manipulate predators. In an attempt to satiate seed predators, newly planted prairie seed was supplemented by a plentiful source of birdseed as an alternative food source. We hypothesized that a ten-fold greater density of supplemental birdseed would satiate the local seed predator community, leading it to consume fewer prairie seeds. We monitored seed removal rates on experimental seed cards (Westerman et al. 2003) as a proxy for seed predation rates in the prairie planting itself. These rates were compared to the actual number of seedlings that establish the first year of the planting. We predicted that this lower rate of predation on prairie seed

would result in: 1) more prairie seed left on experimental seed cards at the time of planting and 2) greater subsequent early seedling establishment. The goal of this experiment was to seek an applicable real world strategy to manipulate seed predators. The use of supplemental seed to reduce predation could provide a practical, inexpensive strategy to improve prairie restorations across the Midwest.

CHAPTER 2

METHODS

Supplemental seed was broadcast immediately following three roadside prairie plantings to determine whether this alternative source of food would reduce predation of native prairie seed. To track predation in the plantings I used two approaches. The first approach was a buffet experiment using seed cards, which attempted to directly measure seed loss due to predation immediately after the planting. The second approach attempted to estimate seed predation of native prairie seed by measuring early native seedling establishment. Early emergence of planted species was tracked the following summer. While it would be most ideal to quantify seedling emergence instead of establishment, accurate identification of these seedlings was not feasible until past the emergence stage. Establishment, which is emergence minus seedling death, is a more accurate term for the timing of our sampling. This metric is still a good measure of the impact of seed predation because it is unlikely that supplemental seed would affect seedling mortality.

Site Descriptions

I conducted my research at three sites that were county roadside rights-of-way. These rights-of-way were regraded during the summer of 2014 and were planted to native prairie vegetation in the fall of 2014 by county secondary roads departments. The experiment included one site in Linn county: (1) Linn (Linn) and two sites in Benton county: (2) Benton North (BN) and (3) Benton South (BS) (Figure 1).

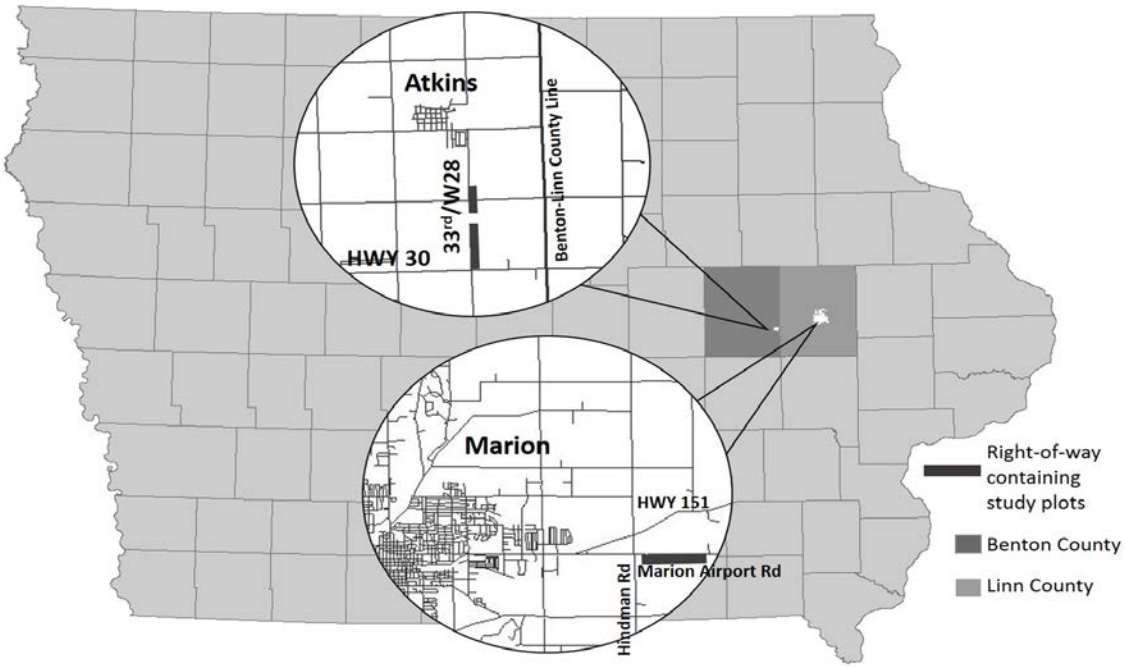

Figure 1: Location of study plots within Benton and Linn counties.

Linn County Site

The Linn County right-of-way (42° 2’ 6.6” N; 91° 31’ 13” W) was located in Marion, IA. The site was 0.11 ha in the right-of-way along Marion Airport Road which runs East/West. The right-of-way is on the south side of the road and the land adjacent was managed for corn production in 2014 and bean production in 2015. The intersecting Hindman Road right-of-way contains prairie vegetation planted in 2008 (Figure 2). Prior to the regrade project, the site was vegetated by mostly nonnative grasses, including *Agropyron repens* L. (Quack grass), *Bromus inermis* Leyss. (Smooth brome), *Medicago* sativa L. (alfalfa), *Poa pratensis* L. (Kentucky blue-grass), and *Trifolium pretense* L. (red

clover). Management consisted of biannual mowing of the shoulder and spot spraying or mowing of noxious weeds. The soil of the Linn site is classified as Klinger-Maxfield silty clay loams. Regrading the right-of-way reshaped or removed an unknown and uneven amount of topsoil, but the subsoil is a silty clay loam through the Bg2 horizon (26 to 33 inches). From the 2Bg3 horizon down to 80 inches, the subsoil is classified as loam (Soil Survey Staff Natural Resources Conservation Service 2013). During the summer of 2014, the existing vegetation and a layer of topsoil was stripped from the site to fill in rights-of-way elsewhere in the county. On August 6, 2014, the site was planted with a cover crop including, *Avena sativa* L. (common oats; 5.60g/m^2), *Elymus canadensis* L. (Canada wild rye; 0.112 g/m^2), and *Secale cereale* L. (cereal rye; 5.60 g/m^2). The cover crop was hydroseeded with water using a Finn T-90 hydroseeder and then cultipacked using a Reinco 6 foot mulch disc cultipacker (Finn Corporation 2015; Reinco 2015). Six plots were established at this site on September 26, 2014 (Figure 2). Each plot was 5 m East/West and 37m North/South, set end to end. Foreslope (North facing) corners and center of the plot were marked with a 25.4cm spike nail and flagging. Backslope (South facing) corners and center were marked with snow poles for visibility. On November 12, 2014, the Linn County Secondary Roads Department drill seeded the site with a 30 species mix at the rate of 1.174 g/m^2 (576.542 seeds/m^2) using a Truax grass drill (Truax Company Inc. 2009; Table 1). During the growing season (6/23/2015) sites were mowed to manage weeds and prevent the cover crop from becoming the dominant vegetation.

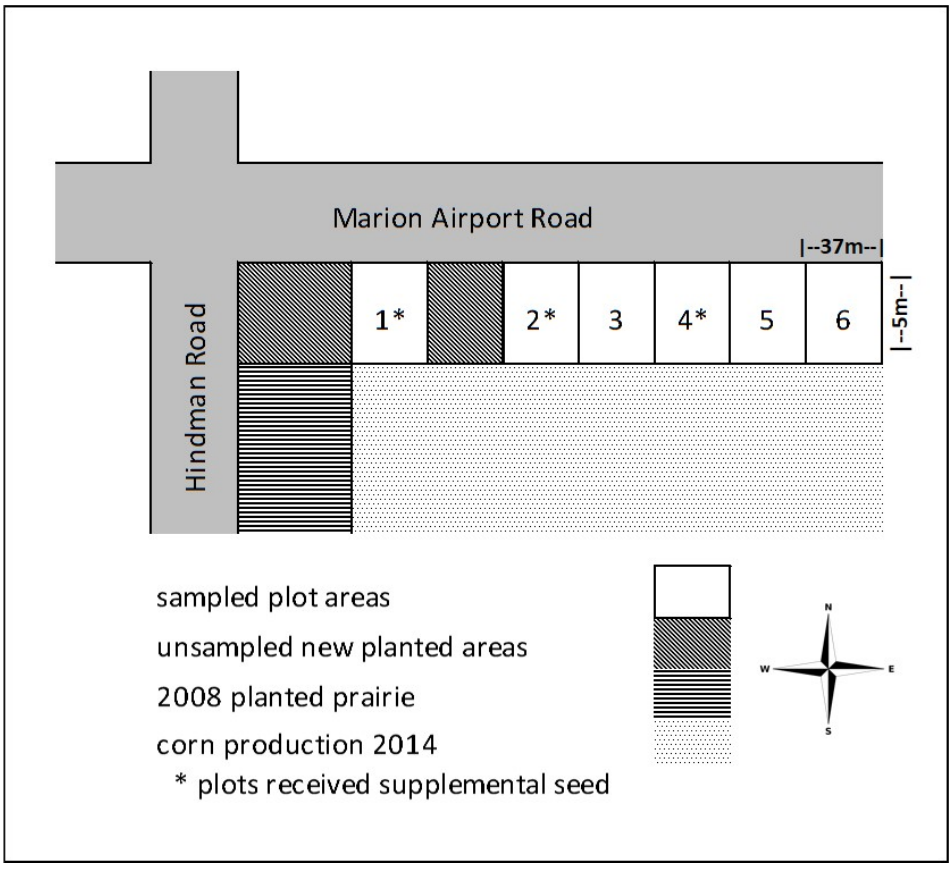

Figure 2: Linn county site layout (not to scale).

First frost date of 2014 at the site was 10/30/2014. The site received approximately 2cm of snow on 11/15/2014, 3 days after the planting. All 3 sites share a common closest weather station near Marion, IA. The average temperature during the duration of the study (from September to July) was 7.8°C, which is similar to the average year. The average temperature during the summer growing season was 19.7°C which is similar to the average year. The average winter temperature was -4.0°C, which is slightly

colder than the average year. The average precipitation per month during the summer growing season was 73.2 mm, which is wetter than average (Figure 3; NOAA 2015).

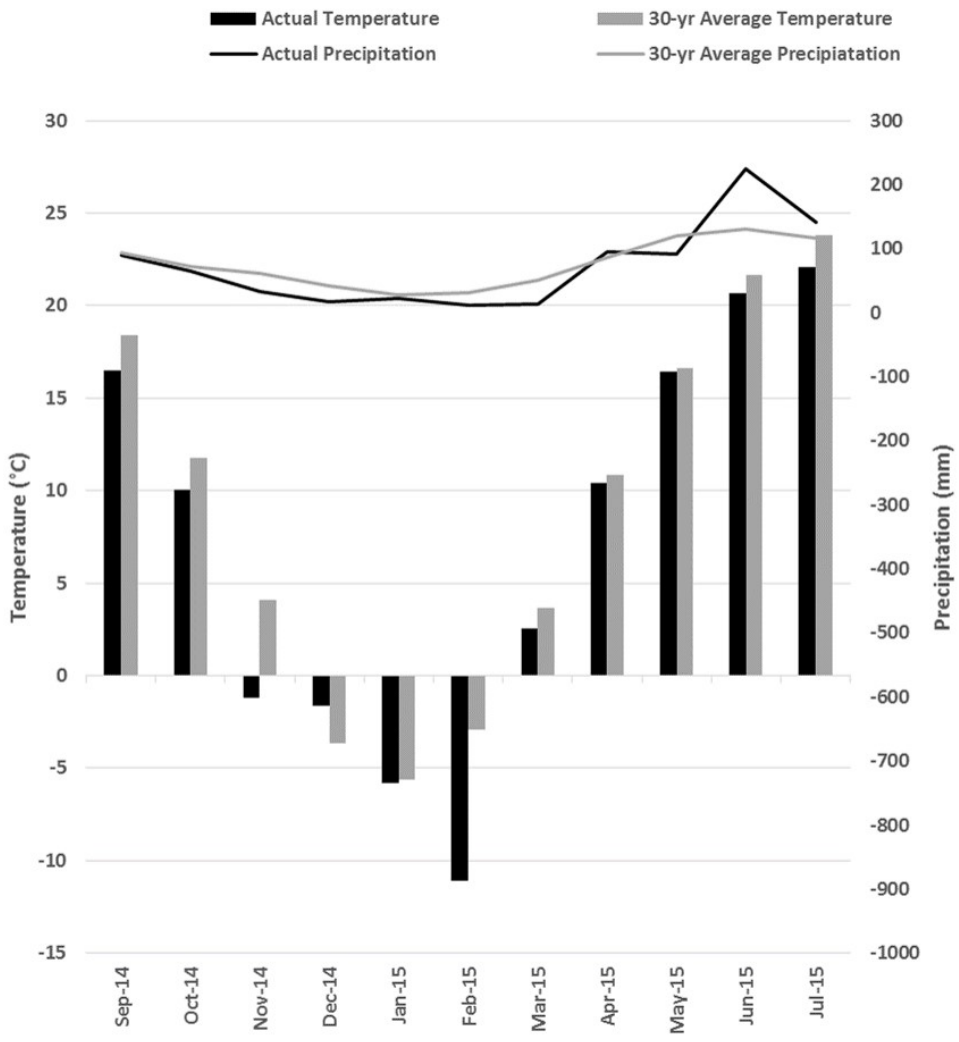

Figure 3: Mean monthly temperatures and precipitation and 30-year average for the 3 sites for the duration of the study period (NOAA 2015).

Table 1: Seed Mixes for Linn and Benton County Sites. Nomenclature from USDA plants database (USDA/NRCS 2015).

		Linn		Benton	
Common name	Scientific name	g/m^2	seeds/m^2	g/m^2	seeds/m^2
Lead plant	*Amorpha canascens*	0.02	9.88	0.07	42.07
Big bluestem	*Andropogan gerardii Vitman*	0.13	47.44	0.57	201.72
Swamp milkweed	*Asclepias incarnata* L.	0.00	0.00	0.09	15.13
Butterfly Milkweed	*Asclepias tuberosa* L.	0.02	2.66	0.07	11.29
Canada milkvetch	*Astragalus canadensis* L.	0.02	10.08	0.07	42.87
White wild indigo	*Baptisia alba (L.) Vent.*	0.01	0.84	0.06	3.57
Sideoats grama	*Bouteloua curtipendula* (Michx.) Torr.	0.13	28.47	0.57	121.03
Prairie sedge	*Carex bicknellii* Britton	0.00	11.68	0.01	49.64
Fox sedge	*Carex vulpinoidea* Michx.	0.00	0.00	0.01	31.52
Partridge pea	*Chamaecrista fasciculata* (Michx.) Greene	0.12	7.41	0.52	31.52
Purple prairie clover	*Dalea purpurea* Vent.	0.02	12.97	0.10	55.16
Showy tick trefoil	*Desmodium canadense* (L.) DC.	0.01	1.63	0.04	6.93
Pale purple coneflower	*Echinacea pallida* Nutt.	0.02	4.50	0.10	19.12
Canada wild rye	*Elymus canadensis* L.	0.11	20.56	0.48	87.41
Rattlesnake master	*Eryngium yuccifolium* Michx.	0.02	5.56	0.09	23.64
Sneezeweed	*Helenium autumnale* L.	0.00	0.00	0.03	136.58
Ox-eye sunflower	*Heliopsis helianthoides* (L.) Sweet	0.03	7.01	0.13	29.79
Round-headed bushclover	*Lespedeza capitata* Michx.	0.01	3.95	0.06	16.81
Rough blazing star	*Liatris aspera* Michx.	0.02	13.84	0.10	58.83
Prairie blazing star	*Liatris pycnostachya* Michx.	0.03	11.42	0.13	48.54
Wild bergamot	*Monarda fistulosa* L.	0.02	43.24	0.07	183.86
Stiff goldenrod	*Oligoneuron rigidum* (L.) Small	0.01	12.16	0.04	51.69
Switchgrass	*Panicum virgatum* L.	0.00	0.00	0.48	235.34
Large-flowered beardtongue	*Penstemon grandiflorus* Nutt.	0.01	5.19	0.04	22.06
Grey-headed coneflower	*Ratibida pinnata* (Vent.) Barnhart	0.02	25.95	0.10	110.31
Black-eyed Susan	*Rudbeckia hirta* L.	0.02	56.83	0.07	241.64
Wild petunia	*Ruellia humilis* Nutt.	0.01	1.54	0.04	6.56
Green bulrush	*Scirpus atrovirens* Willd.	0.00	0.00	0.01	144.99

(table continues)

Common name	Scientific name	Linn g/m^2	Linn seeds/m^2	Benton g/m^2	Benton seeds/m^2
Compass plant	*Silphium lacinatum* L.	0.01	0.20	0.04	0.83
Indiangrass	*Sorghastrum nutans* (L.) Nash	0.13	56.93	0.57	242.06
Rough dropseed	*Sporobolus compositus* (Poir.) *Merr.*	0.13	132.84	0.57	564.81
New England aster	*Symphyotrichum novae-angliae* (L.) G.L. Nesom	0.01	19.57	0.04	83.21
Ohio spiderwort	*Tradescantia ohiensis* Raf.	0.03	7.91	0.12	33.62
Hoary vervain	*Verbena stricta* Vent.	0.01	8.30	0.04	35.30
Golden Alexanders	*Zizia aurea* (L.) W.D.J. Koch	0.02	5.98	0.07	25.42
Mix totals		1.17	576.54	5.61	3014.88

Benton County Sites

The two Benton County sites were rights-of-way along 33rd Avenue/W28, in the portion that runs North/South between Atkins, IA and Highway 30. The northern site (BN) was 0.3 ha (41° 59’ 7” N; 91° 51’ 13” W) and the southern site (BS) was 0.55 ha (41° 57’ 29” N; 91° 51’ 13” W), both on the East side of the roadway. Vegetation East of the right-of-way was managed for soybean production in 2014. Portions of the right-of-way West of 33rd Avenue were planted to native prairie vegetation in the summer of 2013 (Figure 4, Figure 5). Prior to the regrade project, both sites were vegetated by mostly nonnative grass, primarily *Bromus inermis* Leyss. (Smooth brome) and occasionally sprayed with 2-4D or Milestone for noxious weeds. The soil of the Benton sites are classified as Kenyon loam. Regrading the right-of-way reshaped or removed an unknown and uneven amount of topsoil but the soils are classified as loam down to 2C horizon (61-80 inches) (Soil Survey Staff, Natural Resources Conservation Service 2013). During

the late summer/early fall of 2014, the existing vegetation and a layer of topsoil was stripped from the site to make the ditch wider, less steep, and to create a shoulder for the road. On September 8, 2014, the sites were planted with a cover crop of *Triticum aestivum* (winter wheat; 5.60 g/m^2). The cover crop was drilled on bottoms and foreslopes of the right-of-way with a Truax Flex ll drill and hydroseeded with water on the backslopes using a Finn hydroseeder (Truax Company Inc. 2009; Finn Corporation 2015). At Benton North, eight plots were established at this site on September 26, 2014 (Figure 4). Each plot was 10 m wide and 37m long, set end to end. Foreslope (East facing) corners and center of the plot were marked with a 25.4cm spike nail and flagging. The same procedure was followed with the same equipment at Benton South, where 15 plots were established. Backslope (west facing) corners and center were marked with snow poles for visibility. On October 29, 2014, the sites were drill seeded at 5.60 g/m^2 (3014.878 seeds/m^2) by the Benton County Secondary Roads Department (Table 1) using the Truax drill. The backslopes were hydroseeded the same day with died wood mulch and a tackifier (a compound that increases the stickiness of the hydroseeding slurry) at 5.60 g/m^2 using the Finn hydroseeder. The site was not mowed until after the sampling period so the cover crop was still standing at the time of sampling. The first frost date of 2014 at this site was October 23, 2014. The site received snow on 11/15/2014. Though the Benton sites are very similar in terms of proximity, seeding rates, and management, they were treated as different sites due to drastic differences in shape of the ditch profile and hydrology. For example, Benton N

was a much dryer upland site, while Benton North was much flatter, wetter, and muddier most of the growing season.

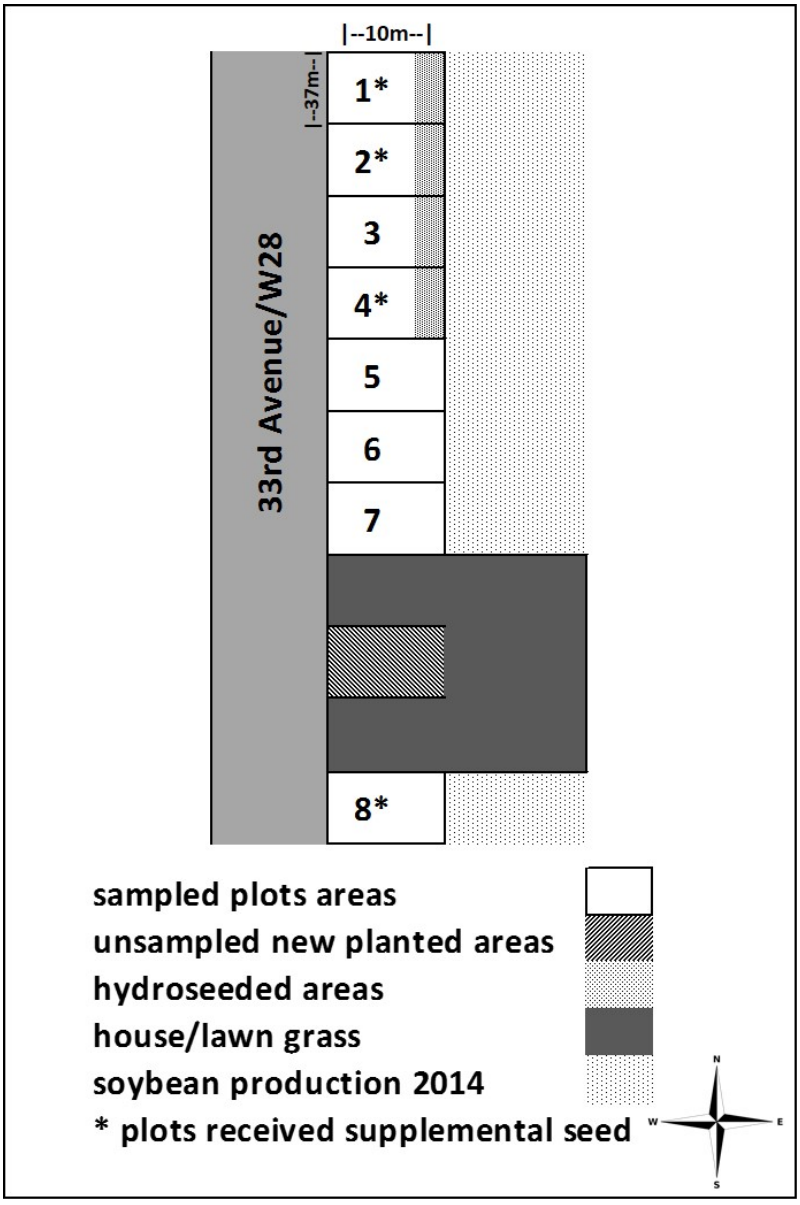

Figure 4: Benton County North (BN) site layout (not to scale).

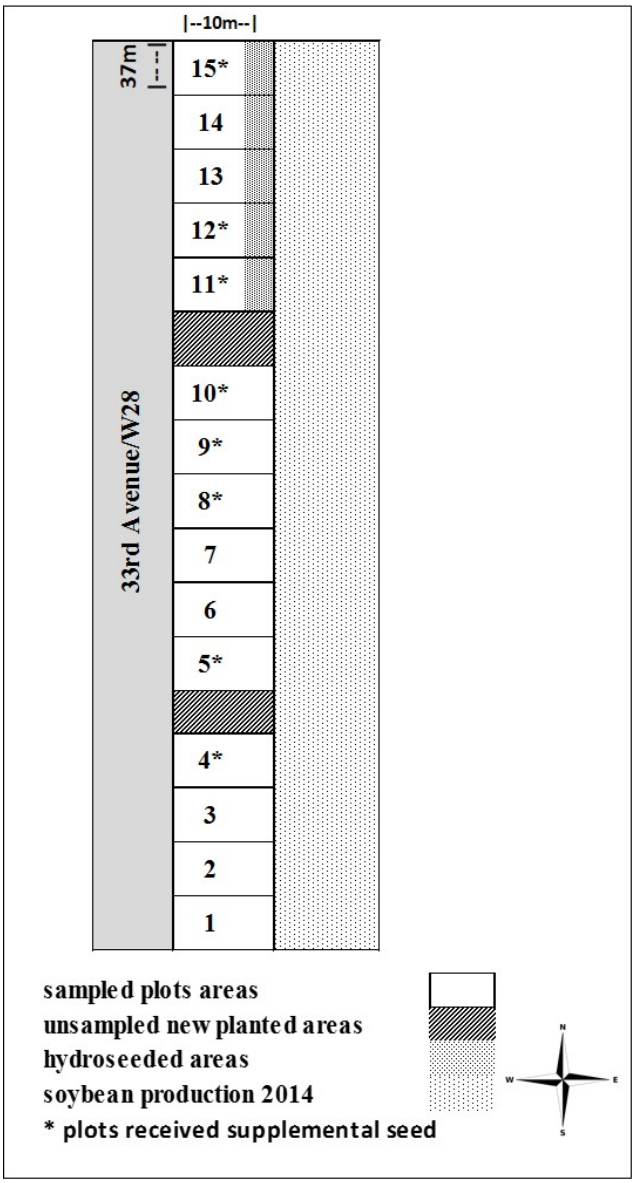

Figure 5: Benton County South (BS) site layout (not to scale).

Supplemental Seed Treatment

This experiment had two treatments, a supplemental seed treatment and a control treatment where no supplemental seed was added to the planting. The supplemental seed mix was comprised of 4 types of birdseed in equal proportions by mass: Nature's Season black oil sunflower (*Helianthus annuus* L.*)*, Nyjer thistle (*Guizotia abyssinica* L. f. Cass.), white millet (*Panicum miliaceum* L.), and Cedar River Milling cracked corn (*Zea mays* L.*)*. The seed was all obtained from Cedar River Milling Company in Waterloo, Iowa. Mineral salt was also added to the supplemental seed mix at the rate of 0.144oz/m^2 (Champion's Choice Mix-N-Fine Mineral Salt). Studies that support mineral/salt lick use by herbivores in order to combat sodium deficiency lend support that salt may increase the palatability of my supplemental food source (Robbins 1983; Weeks and Kirkpatrick 2014). To ensure that the seed would not grow, the sunflower seed and millet seed were roasted at 180°C for 30 minutes. This temperature, 180°C, is enough to kill sunflower seed, so a smaller seed like millet should be killed as well (Corbineau et al. 2002). The thistle seed comes pre-sterilized and the corn is cracked, making the embryos nonviable for germination. We attempted to germinate 32oz of the roasted seed and saw zero germination, confirming that the heat treatment was effective at killing the seed.

This treatment was applied immediately following the drill seeding of prairie seed on planting day. The seed was applied to half of the plots; three plots at the Linn site, four plots at Benton North, and eight plots at Benton S, at ten times the seeding

rate of prairie seed (Table 2). The supplemental seed was applied to all parts of the plots (foreslope, backslope, bottoms) using a hand broadcast seed spreader (PlantMates model 76300).

Table 2: Plots and seeding rates at each site.

Site	Number of Plots	Number of treatment Plots	Seeding rate of Prairie seed	Seeding Rate of supplemental seed
Linn	6	3	1.174 g/m^2	11.74 g/m^2
Benton N	8	4	5.60 g/m^2	56.00 g/m^2
Benton S	15	8	5.60 g/m^2	56.00 g/m^2

Seed Removal Experiment

The seed removal experiment was a buffet experiment, where the rate that seed was removed was a proxy measure for seed loss immediately after the roadside planting. The experimental design was a randomized block using the three sites (Linn, Benton N, Benton S) as blocks, each containing a variable number of plots (6, 8, and 15 plots). The experiment had two treatments: supplemental seed and control (no supplemental seed).

Seed loss was assessed by monitoring the removal from 'seed cards,' which were seeds attached to squares of coarse sandpaper. Each plot received seven seed cards as sub-replicates, plus one control card. The seven cards were placed along a transect

through the center of each plot, but spaced along the width so that there were cards along the whole profile of the slope (Figure 6, Figure 7). The control card was placed inside a metal cage (32cm x 14cm x 8cm) to exclude vertebrate predators and then the metal cage was placed inside a bag made of insect barrier cloth (Agribon + AG 15, 118'' X 50', lightweight grade) to exclude invertebrate predators (Figure 8, Figure 9). Both the metal cage and the cloth bag were closed with twist ties so that they could be reopened to count the card and then reclosed. The caged control cards were used to measure ambient loss of seed due to factors other than predation such as wind, rain, failure of the adhesive or other flaws in card design. In each plot, the cage was randomly assigned to one of the 7 seed card positions and placed next to the card at that position. Rocks were placed on the bags to keep the cages from blowing away.

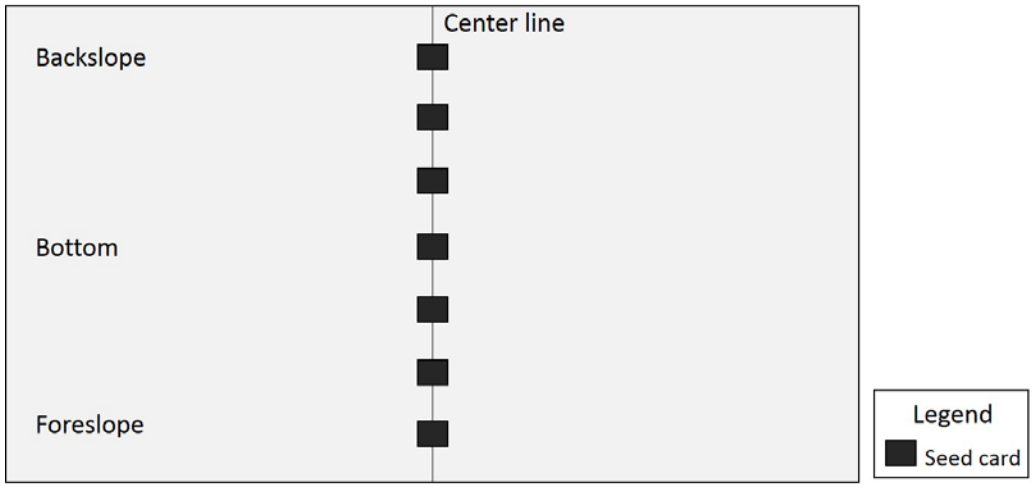

Figure 6: Schematic diagram of seed card placement. The box indicates the entire plot, 37m long running parallel to the road. Cards were placed equidistant to one another, along the center line of each plot.

Figure 7: Cards were placed equidistant to one another, along the center line of each plot. The card used to measure non-predation seed loss was randomly placed next to one of the 7 cards.

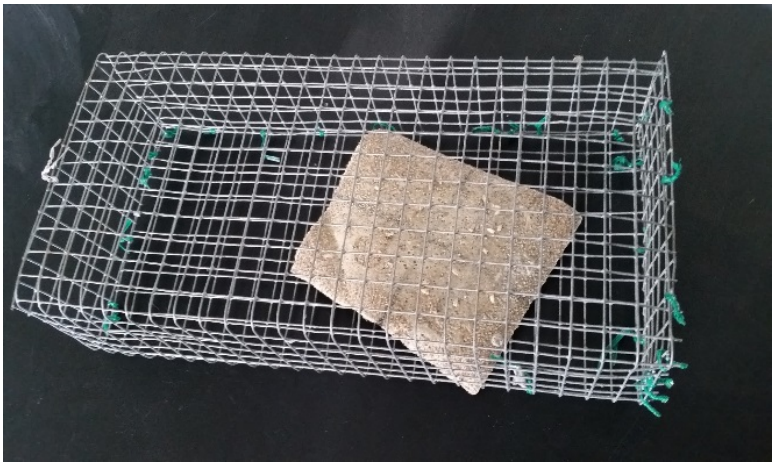

Figure 8: Control card in metal cage.

Figure 9: Cage inside insect barrier cloth bag

Construction of the seed cards was adapted from a method described in Westerman *et al.* (2003). We used a high quality sand paper (3M Paper Sheet 346U, 36 Grit, aluminum oxide commercial D-weight) cut into fourths (11 cm x 14 cm) and attached the seeds with multiple layers of aerosol spray adhesive (3M Super 77 Multipurpose Adhesive Aerosol; Figure 10). First, a base layer was applied to the card. Then, seeds were placed on the card and a top layer was applied. The second top layer was applied 24 hours after the first layer to ensure that the glue dried between applications. The strength of the glue ensured that the seeds stayed on during rain, snow, or other weather conditions, while still enabling predators to remove the seeds. Cards were labeled with a permanent marker and allowed at least 48 hours to dry before being placed outside. Roofing nails were used to secure the seed cards to the ground (Grip Fast 2-inch Electro-Galvanized Roofing Nail).

Thirty well-filled seeds of *Echinacea pallida* were glued to each seed card, randomly scattered into 4 quadrants. This species was chosen for its size and shape (relatively small, yet manageable to work with and identify for counting) and because it was included in the seed mix for all of the planting sites. Quadrants were used to increase accuracy of counting the seeds. According to Westerman, there was no evidence of the glue or sandpaper causing attraction, avoidance, or disturbance to the seed cards by the seed predators (2003).

Figure 10: Seed card design showing sandpaper, divided into four quadrants, and seeds between layers of aerosol adhesive.

Two sets of seed cards, control cards, and insect bags were prepared as data on this experiment was collected twice. First, a week-long pre-planting trial was conducted to assess background predation rates and the second time, a 2 week post-planting count was conducted immediately following the planting (Table 3). A 0°C frost occurred between the pre-planting count and planting count at all of the sites, which would have killed insects. This means that the pre-planting count had both vertebrate and invertebrate predators present, while the planting count only experienced vertebrate predators. On the first day of each trial, cards were placed at the sites. Cards were counted midway through the week and again on the 7th day. For the second set, data was also collected on day 14. On the last day, cards and were removed from the site. On

each counting day we counted the intact seeds on each card (including control cards) to see how many seeds remained. Partially-consumed or damaged seeds were removed before counting and a seed was only counted if the whole seed (embryo) was intact. Seeds that were missing, chewed open, broken, crushed, or damaged in any way were considered consumed. Day 7 pre-planting cards were compared to day 7 planting cards from only the control treatment.

Table 3: Timeline of seed removal experiment.

Site	Start/End of Seed Card Count (Pre-planting)	First frost date	Planting Date	Start/End of Seed Card Count (Planting)
Linn	9/26/14 - 10/3/14	10/30/14	11/12/14	11/12/14 - 11/26/14
Benton N	9/26/14 - 10/3/14	10/23/14	10/29/14	10/29/14 - 11/12/14
Benton S	9/26/14 - 10/3/14	10/23/14	10/29/14	10/29/14 - 11/12/14

Early Seedling Establishment Experiment

From July 20-24, 2015, two teams of two people each counted established seedlings at the three sites. Data taken from each plot included: number and species identification of seedlings, basal area measurements (for all seedlings, forbs, grasses, sedges, weeds, and cover/nurse crops), and the degree of both slopes. Prior to sampling, sites were divided into three sections of the ditch profile: foreslope (the section next to the road), bottom, and backslope (the section next to the farm field)

(Figure 11). The bottom was defined as having 0% slope. The ditch was marked as foreslope or backslope when the slope was greater than or equal to five degrees as determined by a clinometer. Five, 0.1-m^2 quadrats were sampled in each of the three sections of the ditch profile (fifteen per plot; Figure 12). All sampling occurred in the center five meters of the plot to minimize variation and allow the sampling area to be buffered from the adjacent plot by the rest of the plot. Within the five meter area, the quadrats were placed at five random positions down the length of the plot, in the middle of the ditch section (by width). Placing the quadrat in the middle ensured that it sat completely within the intended ditch section. All seedlings from the planted seed mix were counted and identified according to Iowa seedling ID guides, but the two *Liatris* species and the two *Carex* species were pooled as "*Liatris* species" and "Carex species" to avoid misidentifying similar species (Natural Resources Conservation Service 2005; Williams 2010). Any species not planted according to the seed mix was counted as a weed species.

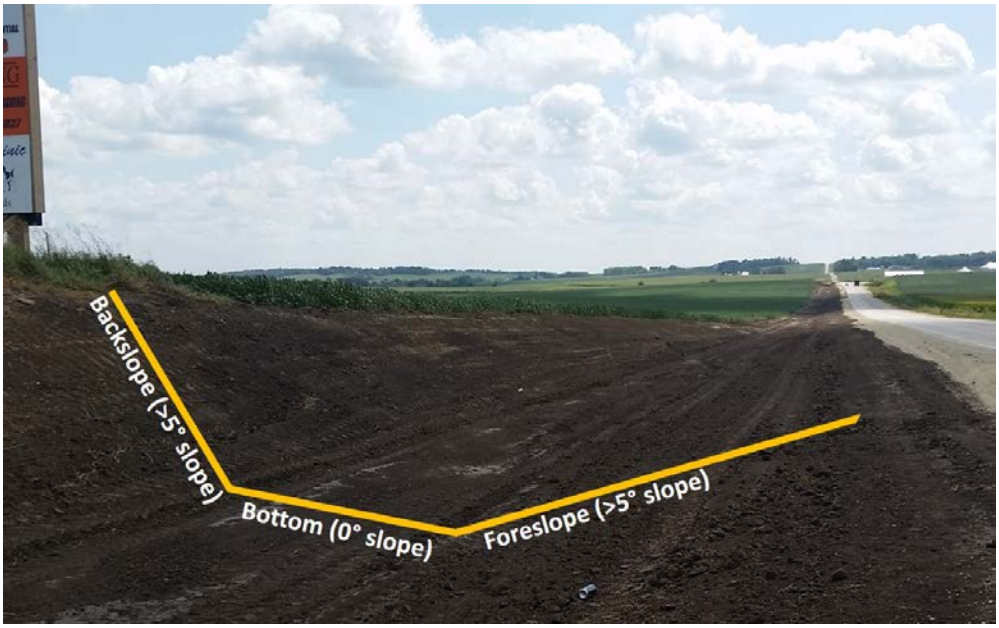

Figure 11: Sections of the ditch profile (Backslope, Bottom, Foreslope).

	← 16 m more plot	Center 5m Sampling Area	16 m more plot →
Foreslope		X X X X X	
Bottom		X X X X X	
Backslope		X X X X X	

Figure 12: Placement of quadrats for early seedling establishment. Quadrats were placed at 5 random positions down the length of the plot, in the middle of the ditch section (by width).

Data Analysis

For the seed card experiment, the number of remaining seeds per card was used to calculate a mean for the 7 cards in each plot. Control cards showed a loss of less than 1% of seeds so cards were not corrected for passive losses. The effect of the supplemental seed treatment versus the control treatment on the amount of seeds remaining was tested using a mixed effects general linear model. In the model, treatment is a fixed effect and site is a random effect, taking into account variation between the 3 sites. An interaction term (site*treatment) was removed from the analysis after it was found to be non-significant. We also tested the effect of trial (pre-planting vs. planting) on the amount of seeds remaining using a similar linear mixed effects model that accounted for repeated measures. For the early seedling establishment experiment, the number of established seedlings from each of the five, 0.1-m^2 quadrats from each section of the ditch profile, were added together and then divided by 0.5 to calculate mean seedlings per m^2. The effect of the supplemental seed treatment versus the control treatment, the effect of site, and the effect of section of the ditch profile were tested using a general linear model.

In all cases, data sets were visually inspected for normality using boxplots and scatter plots (qqnorm plots) of model residuals-versus-predicted values for each analysis. When data were non-normal, the Box-Cox function was used to transform the data. All data were analyzed using the program R, package version 3.1-109 (R Core Team 2013) and the package ‘nlme’ (Pinheiro et al. 2013).

CHAPTER 3

RESULTS

Seed Removal

In the first 7 days after the planting and treatment application day, predators had removed 4.3% of seeds from the experimental cards and by day 14, they had removed 7.4% of the seeds. Predators removed 8.4% of seeds from the seed treatment plots and 6.2% from the control treatment plots, but the plots did not significantly differ by treatment ($p > 0.05$; Table 4, Table 5). According to these results, adding supplemental seed did not significantly reduce predation on prairie seeds at the time of seeding.

Though there was not a significant effect of the supplemental seed treatment, there was a significant difference between the two seed card trials. By the 7th day of the pre-planting trial (which occurred before a 0°C frost), predators removed 24.8% of seeds from the experimental cards compared to the 4.3% that were removed during the planting trial (which occurred after a 0°C frost; $p<0.001$; Table 4, Table 6).

Table 4: Mean seeds removed (out of 30) in the control and seed treatments on day 14 and the pre-planting and planting trials on day 7. See Table 3 for sampling dates.

	mean seeds removed	Standard error	predation rate
control	1.86	0.41	6.20%
seed	2.53	0.48	8.44%
pre-planting	7.44	0.94	24.80%
planting	1.29	0.31	4.30%

Table 5: Linear mixed effects model ANOVA for seeds remaining in the supplemental seed treatment versus the control treatment at all sites after 7 days.

	Numerator DF	Denominator DF	F-value	p-value
(Intercept)	1	25	1916.2168	<.0001
treatment	1	25	1.3321	0.2593

Table 6: Linear mixed effects model ANOVA for seeds remaining in the planting versus pre-planting trials at all sites.

	Numerator DF	Denominator DF	F-value	p-value
(Intercept)	1	12	1253.5302	<.0001
trial	1	12	27.8528	<0.00

Early Seedling Establishment

In the growing season that followed the fall planting, a mean of 142.02 ± 12.86 native seedlings/m^2 (mean ± SE) established across all three sites, which is 9.21% of all seeds planted. Mean seedlings established ranged from 103.56 to 184.17 seedlings/m^2 at the different sites and the effect of site was significant ($p<0.005$; Table 7). Mean early establishment rates for each species individually is summarized by site in Table 8. The

Benton North site had significantly more early seedling establishment (184.17 ±32.3) than the Linn site (103.56 ±29.35), but neither the Linn site, nor the Benton North site were significantly different than the Benton South site (107.24 ± 11.03); (Figure 13). Keeping in mind that Benton was seeded at almost five times the rate of Linn County (5.60 g/m^2 vs. 1.17 g/m^2), Linn had the greatest percent establishment at 17.96%, followed by Benton North at 6.11% and Benton South at 3.56%.

Table 7: Linear model ANOVA for early seedling establishment at all sites.

Source	Df	SS	MS	F	p
Site	2	104557	52279	6.0959	0.003626 **
Treatment	1	69950	69950	8.1564	0.005643 **
Section of ditch profile	2	194671	97336	11.3497	5.379e-05 ***
Site x Treatment	2	29157	14579	1.6999	0.190150
Treatment x Section	2	8697	4349	0.5071	0.604448
Site x Section	4	145990	36498	4.2558	0.003854 **
Residuals	70	600322	8576		

Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table 8: Mean early seedling establishment/m² and standard error (se) by species. Species are ordered from most common to least common across all sites.

	overall		Linn		Benton N		Benton S	
species	mean	se	mean	se	mean	se	mean	se
Rudbeckia hirta	18.18	2.70	27.67	4.45	24.13	4.48	10.71	1.96
Echinacea pallida	11.79	1.30	4.67	0.87	14.50	2.83	13.29	1.71
Sorghastrum nutans	9.79	2.04	4.44	0.70	21.17	6.11	5.57	1.40
Verbena stricta	9.52	1.31	4.33	0.59	13.25	3.02	9.62	1.80
Heliopsis helianthoides	7.79	0.79	4.22	0.69	10.50	1.89	7.76	0.87
Penstemon grandiflorus	7.71	1.16	1.33	0.41	10.75	2.54	8.71	1.62
Ratibida pinnata	7.00	1.29	13.22	1.53	16.25	2.97	6.67	1.07
Andropogan gerardii	6.79	1.22	3.89	0.78	13.42	3.07	4.24	1.27
Eryngium yuccifolium	6.36	0.83	1.22	0.20	9.00	2.27	7.05	0.85
Bouteloua curtipendula	6.24	1.01	4.67	0.79	7.58	1.94	6.14	1.54
Zizia aurea	5.05	0.61	5.67	0.59	4.58	1.14	5.05	0.88
Ruellia humilis	3.29	0.46	0.44	0.12	4.75	1.15	3.67	0.56
Sporobolus compositus	3.02	0.87	8.44	1.69	1.83	0.60	1.38	0.46
Carex sp.	2.86	1.18	3.00	0.49	7.50	3.95	0.14	0.08
Monarda fistulosa	2.57	0.47	1.78	0.40	3.58	1.17	2.33	0.55
Tradescantia ohiensis	2.38	0.41	0.00	0.00	1.58	0.40	3.86	0.71
Desmodium canadense	2.24	0.32	1.22	0.19	3.17	0.81	2.14	0.38
Elymus canadensis	2.24	0.47	4.89	0.84	1.50	0.35	1.52	0.42
Dalea purpurea	2.14	0.43	0.89	0.20	4.92	1.19	1.10	0.34
Liatris sp.	1.60	0.30	1.00	0.29	1.58	0.71	1.86	0.36
Lespedeza capitata	1.50	0.27	1.33	0.21	2.33	0.72	1.10	0.28
Asclepias incarnata	1.10	0.22	0.00	0.00	1.92	0.58	1.10	0.24
Oligoneuron rigidum	0.76	0.15	1.00	0.13	0.92	0.38	0.57	0.18
Asclepias tuberosa	0.69	0.18	0.89	0.15	0.75	0.43	0.57	0.22
Chamaecrista fasciculata	0.67	0.16	2.00	0.21	0.67	0.31	0.10	0.07
Astragalus canadensis	0.52	0.14	0.67	0.15	0.92	0.38	0.24	0.10
Baptisia alba	0.31	0.10	0.00	0.00	0.58	0.28	0.29	0.13
Amorpha canascens	0.21	0.11	0.44	0.21	0.17	0.12	0.14	0.08
Panicum virgatum	0.14	0.08	0.00	0.00	0.25	0.14	0.14	0.14
Helenium autumnale	0.10	0.05	0.00	0.00	0.17	0.12	0.10	0.07
Symphyotrichum novae-angliae	0.05	0.03	0.11	0.05	0.00	0.00	0.05	0.05
Scirpus atrovirens	0.02	0.02	0.00	0.00	0.00	0.00	0.05	0.05
Silphium lacinatum	0.02	0.02	0.11	0.05	0.00	0.00	0.00	0.00

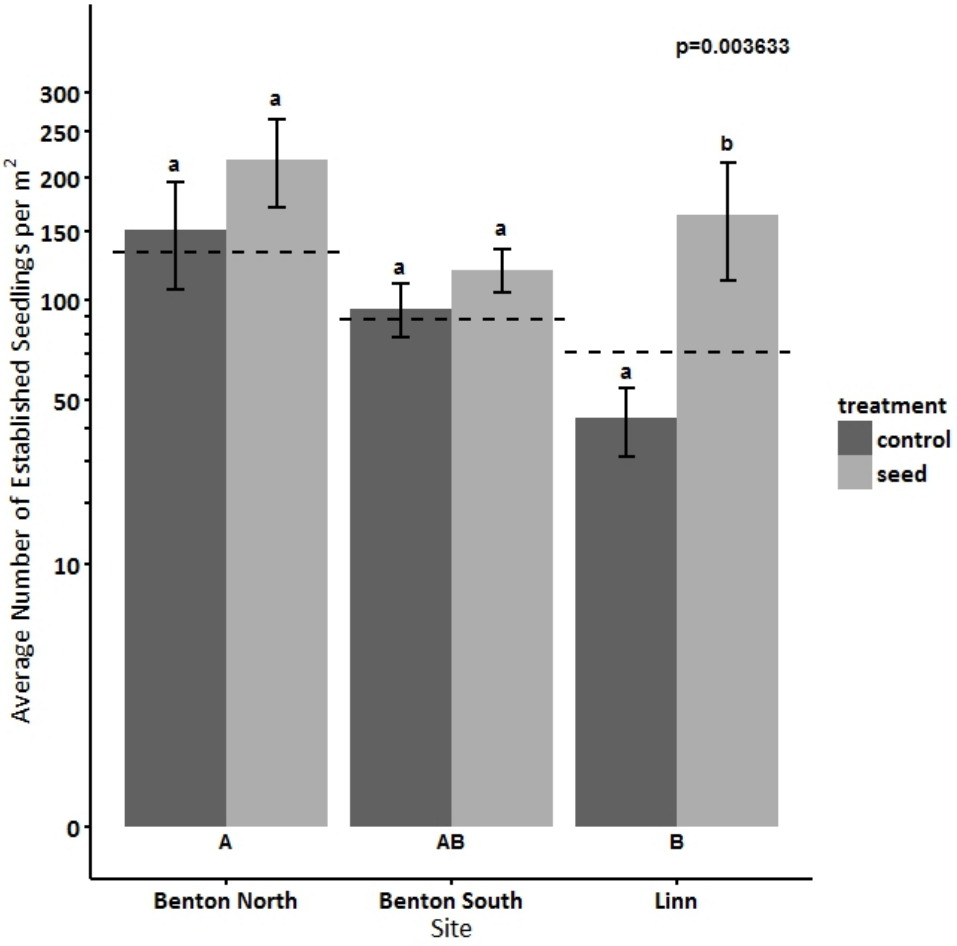

Figure 13: Mean early established seedlings per m^2 at each of the three sites by treatment. P value represents the main effect of site. Values are means +/- standard error. Lowercase letters represent differences between treatments within each site. Uppercase letters represent differences between sites.

Across all three sites, significantly more seedlings established in the plots that received the supplemental seed treatment (157.29 ± 19.33) compared to the control treatment (99.57 ± 15.99); ($p < 0.001$; "All Sections" Figure 14, Table 7). This is a difference of 36.7% more seedlings in the supplemental seed treatment plots than the

control plots. At each of the sites individually, mean early seedling establishment was always greater in the supplemental seed treatment plots than the control treatment, ranging from 21.4-73.7% more, though this difference was only significant at Linn County ($p<0.005$). In each of the three sections of the ditch profile, mean early seedling establishment was always greater in the seed treatment plots than the control plots, ranging from 18.7-59.7% more. This difference was significant in the bottom ($p<-0.05$) and backslope ($p<0.05$), but not the foreslope ($p>0.05$; Figure 14). When species were analyzed by guild, there were significantly more seedlings in supplemental seed plots than control plots in both forbs (Table A2) and grasses (Table A3). There were not enough sedges established to analyze the sedge guild independently.

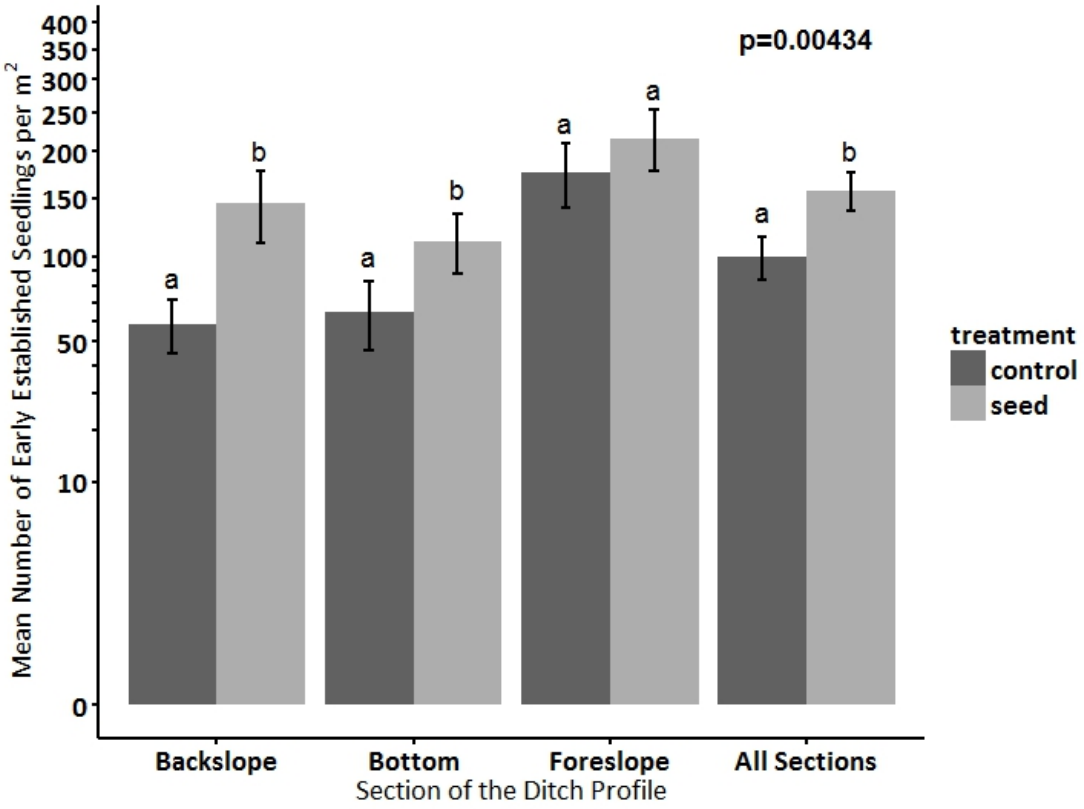

Figure 14: Mean number of early established seedlings per m^2 in the control and supplemental seed treatments for the whole plot (all sections) and each of the sections individually. P-value represents the main effect of treatment (all sections). Values are means +/- standard error.

I also observed a significant difference in early seedling establishment between the three sections of the ditch profile across all sites (p=8.55e-05; Table 8; “All Sites" Figure 15). With a mean of 87.93 seedlings/m^2, the bottom had fewer seedlings than the backslope at 101.29 seedlings/m^2 and the foreslope at 196.07 seedlings/m^2. Both the bottom and backslope differed significantly from the foreslope, but not from each

other. I also observed a significant interaction between site and section of the ditch profile (Table 8; Figure 16). This interaction shows that at Benton South and Linn the backslope and foreslope were not different from one another, but at Benton North, where there was very high foreslope establishment, they are significantly different from each other (Figure 15). In addition, the interaction shows that there are generally a low number of seedlings in the bottoms, but at Benton North establishment in the bottoms was higher. However, at each individual site the bottom is never significantly different from the backslope (Figure 15).

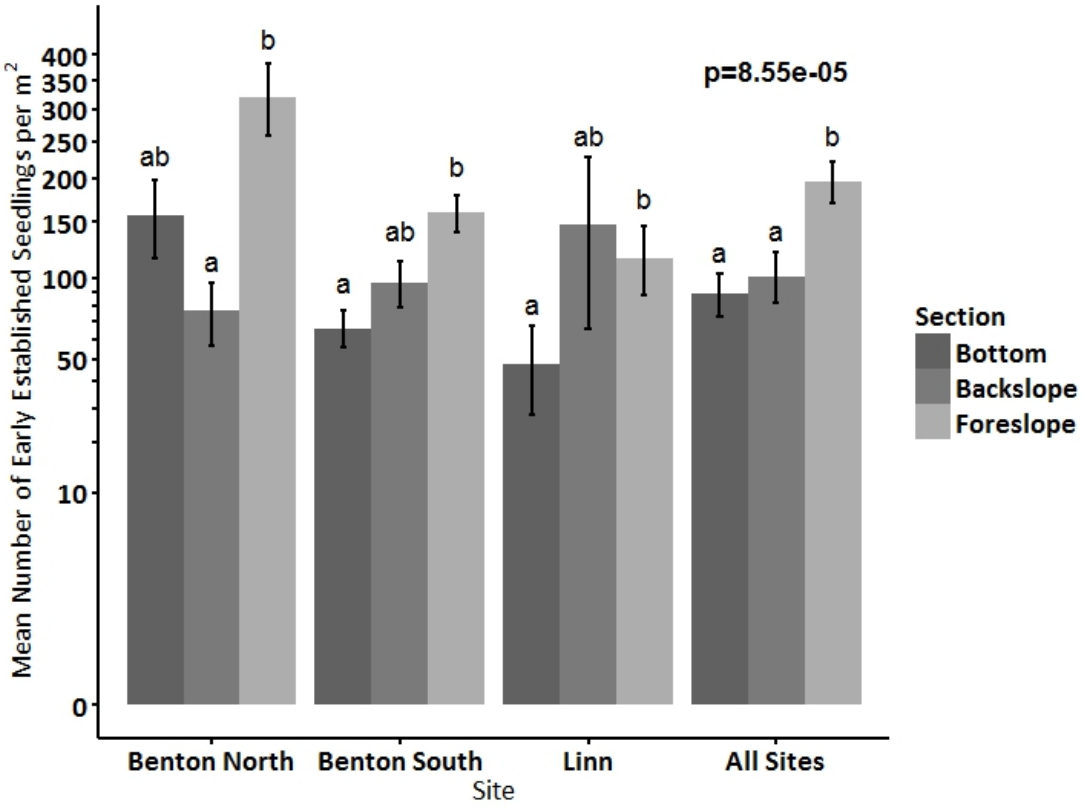

Figure 15: Mean number of early established seedlings per m^2 in each section for all sites combined and each site individually. P-value represents main effect of section. Values are means +/- standard error.

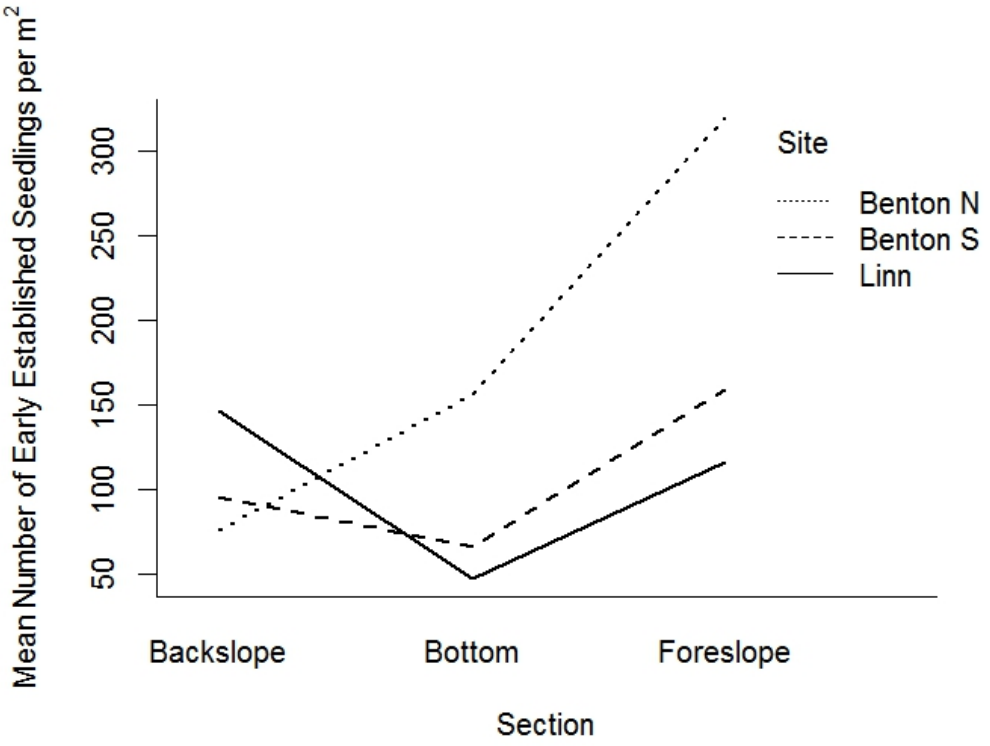

Figure 16: Interaction of site and section of mean early established seedlings per m^2

Additional Results

Basal area of seedlings established differed significantly by site, treatment, and section of the ditch profile (Table A4). In addition, there was a significant interaction between site and section of the ditch profile and a significant correlation to basal area of cover crop. As the amount of cover crop increased, the basal area of established seedlings declined.

CHAPTER 4

DISCUSSION

Other studies have shown high removal rates of seed from seed cards, while others have shown higher seedling establishment when seeds are protected from predators, but until now, these things have not been brought together. The goal for this study was to explore a method of protecting seed in new roadside prairie plantings by satiating predators, thus reducing seed predation in this system. The first approach of measuring predation, using seed removal from seed cards as a proxy for seed predation, showed that not much predation occurred at the time of planting and a treatment effect was not observed. However, the second approach, which measured early seedling establishment, showed that protecting seeds with a supplemental seed source increased early seedling establishment by 37%. The results of this satiation approach presents a potential way of increasing restoration successes and provides the basis for further research.

Based on the hypothesis that seed predators limit seedling establishment and can be satiated by another source of high quality food, I predicted that a ten-fold greater density of supplemental seed would satiate the local predator community. This would lead them to consume fewer prairie seeds, which would result in greater seedling establishment in the first growing season. A statistically significant effect was detected between the plots that received the supplement and those that did not, supporting this prediction.

At ten times the rate of prairie seed, the supplemental seed substantially increased the amount of seed present at planting time compared to a “normal” planting. The results of this study are in line with other studies looking at planting density, particularly weed science studies that suggest that high seed densities may overwhelm consumers’ ability to change plant populations (Pusenius and Ostfeld 2002; Westerman et al. 2003; Cardina et al. 2011; Crawley and Edwards 2011). Our results are also consistent with the evolutionary principle of masting, where there is lower overall seed loss in years of high seed production, when predators are satiated (Janzen 1971). Optimal diet theory is another possible explanation for the results as we observed predators choosing to eat an abundant, higher value prey (birdseed). Higher establishment of prairie plants in treatment plots suggests that there was less predation on the less abundant, lower value prey (small prairie seed) (Janzen 1971; Sih and Christensen 2001). The supplemental seed source chosen for this study was highly palatable and higher in caloric content than the prairie seed. Predators could have chosen to eat this high energy food at a greater rate than the prairie seed for the sake of handling efficiency (Erasmus and Kerley 2011).

I predicted that addition of supplemental seed would result in more prairie seed left on experimental seed cards at the time of planting; however, I did not observe a significant treatment effect. Removal of seeds from seed cards is a proxy for predation rate, so it is possible that this is an unreliable method, though it has been used successfully in several other studies (Westerman et al. 2003; Heggenstaller et al. 2006).

In addition, the cards were definitely accessible to predators, as I made direct observations of grasshoppers, crickets, and ants on and around the cards. However, the extent to which seed loss data from buffet experiments can be extrapolated to reality is debated. If buffet experiments truly overestimate predation rates, the true rate of seed predation at my sites was likely very low (Forcella 2003).

It is also reasonable that a treatment effect was simply not detectable as the overall seed removal rate (7.37% by day 14) was very low at the time the seed cards were deployed, late fall. This is compared to other studies in the tallgrass prairie ecosystem, which have seen loss of up to 66% in the first few weeks, and old field studies which record losses as large as 19.5% within a day of the planting (Mittelbach and Gross 1984; Hemsath 2007b). It is well known that predation is highly variable, even within an ecosystem, but this still begs the question of why predation was so low at my study sites during this time period.

Low predation could be a temporal factor, linked with the type of consumer present. I observed a significantly higher amount of predation occurring in the pre-planting trial of this study than in the planting trial, where a killing frost occurred between the two. Invertebrate predators would have been present for the pre-planting trial, killed by the frost, and thus, not present for the planting trial. We know that the seasonality of seed predation is highly variable, even within the same system, the effect of insect predators in roadsides or prairie ecosystems is unknown. The practice of drilling after a frost is already common practice for some Iowa roadside managers as

they have observed successful first year growth with this method (Rob Roman, personal communication, January 2015), though there does not appear to be studies that have analyzed this directly. Quantifying the presence and impact of insect seed predators in this system would be a logical next step. If insects truly are the major predator in this system, timing plantings after frosts to avoid this suite of predators could contribute to a more quality restoration. The success would depend on the quantity of other types of predators present after frosts as well.

A reduction in seed removal after a killing frost may suggest that invertebrates are strong predators, or that mammal predators were not major predators in fall 2014. Other studies show mammals as major predators in grasslands, agricultural fields, and in forest systems (Heithaus 1981; Heggenstaller et al. 2006; Hemsath 2007b). Some even suggest that rodents are the biggest predator in the fall and winter, when this study was conducted (Westerman et al. 2008; Baraibar et al. 2009; Cardina et al. 2011). However, the sites in this study are dissimilar from other studies of mammal predation in that they provided little to no vegetative cover. Re-grading of the rights-of-way prior to the planting left bare soil, fully stripped of vegetation or litter. The surrounding land was also largely void of cover, as the adjacent agricultural fields were tilled and barren of vegetation for most of the duration of this study. Nurse crops at the study sites would have been the only source of vegetative cover available in late fall 2014. This makes the results of this study more similar to agricultural studies, where predators remove less seed from fields that are bare due to tillage and where more vegetative cover is

associated with increased seed loss (Heggenstaller et al. 2006; Baraibar et al. 2009). In a tallgrass prairie study, predators removed more seeds where there was less litter, but these sites had vegetative cover. Vegetative cover in itself is beneficial to predators, but so are the services that the cover provides, including protection from carnivores, temperature regulation, and nesting sites (Baraibar et al. 2009). These services would have been absent from my study sites, as well as most of the surrounding habitat. Using the seed cards did not capture a treatment effect, but it did allow for a look at the temporal factors (eg. the pre-planting versus planting trials) influencing predation. A significant treatment effect was observed in the seedling establishment experiment, so seed predators must have been active at some point between the late fall seeding and July. It is possible that there was a large predation event that occurred a different time of year (perhaps rodents under the snow, or in the spring when invertebrates emerge) when the seed cards were not in use. Alternatively, predation could occur at a low rate over the entire winter and spring, resulting in a collectively large amount of predation. Using seed cards in future studies at more times of the year may help to capture a fuller picture of predation in the roadside ecosystem.

Limitations and Advantages of the Experimental System

Not being able to directly measure predation was a limitation of this study, though we used methods that are standard for predation studies. Other limitations of this study include fewer replicates than desirable, and great variability between sites. As this study was conducted in new prairie restorations that were conducted by an outside

organization, size of the study area and management decisions were often out of the researcher's control. This, along with maximizing the size of the plots to account for small mammal home ranges and the physical ability to conduct studies on large areas of land, meant that there were fewer replicates per site than desirable for a field study. Not being able to make all management decisions meant that this study included sites that were planted with different seeding rates, at slightly different times, with different frequencies of first year mowing. The roadside system in itself is a highly variable, disturbed system, making it a much more difficult place for an experiment than a restoration in a field site. Sites have three different slopes, affected differently by sunlight, moisture, cover crop establishment, and disturbance by cars, tractors, or snowplows. When possible, efforts were made to minimize these sources of variability. Despite all the variation, a treatment effect was still detected.

Another level of establishment that this study was not able to reach was community structure. Though I identified every species' establishment rate (Table C), the experiment was not designed to detect treatment differences on a by-species basis. Planting density differed by site and seed mixes are designed with different amounts of each species based on price, soil type, and a number of other factors. With this setup, there is little way of knowing whether species A was more abundant than species B because the treatment worked better on species A or because species A was planted at a higher rate. I could normalize these data in order to analyze a treatment effect, but would likely have very low statistical power. While this study showed higher

establishment overall, an experiment with an equal seeding rates across sites and an equal amount of each species in the seed mix would allow for analysis of the treatment's effect on each species individually.

Another unknown in this study is how the mineral salt affected the palatability of the seed mix. Did predators choose to forage on the birdseed because it was higher value prey itself, or because of the salt? Despite not being able to determine this answer, the treatment was successful with the mineral salt application. Future studies could test the use of supplemental seed without a salt addition to judge the effectiveness of the birdseed alone.

Though roadsides are a difficult place to conduct experimental studies, the advantage is that the results directly reflect the study system. The results of this study are a real picture of predation and prairie restoration in roadsides, rather than field experiments extrapolated to the roadside system. This is especially important considering that predation in regraded roadsides may be more similar to predation in tilled agricultural fields than prairie restorations in fields, though it is reasonable that supplemental seed treatments could work in field restorations too. Another advantage to doing a study directly in the roadside system is that despite the great variability, this study still showed a significant treatment effect across all sites. Being able to detect a difference between the treatment and control plots with a lot of variation present, adds support for the use of supplemental seed.

Implications

In this study, seed cards showed that predation did not occur to a large degree at the time of planting, but differences in early seedling establishment suggest that predators that were present later on, could be manipulated by a source of supplemental seed. To our knowledge, this is the first study that has sought to manipulate seed predators (without using exclosures) in the tallgrass prairie ecosystem and successfully reduced predation. Being able to increase early seedling establishment with this method is a huge benefit to new prairies themselves, but could also be an economic benefit to roadside restoration programs. At the recommended rates of prairie seed and supplemental seed (1.174g/m^2 and 11.74g/m^2 respectively), the IRVM diversity mix costs $810.73/ha and supplemental seed cost $129.39/ha. This makes the cost of seeding with this treatment $940.11/ha, a 13.7% increase in cost compared to seeding without the treatment. As seed cost is already a prohibitive part prairie reconstruction, it would be beneficial to be able to use this treatment to achieve high establishment at a lower cost. In this study, implementing this treatment (spending 13.7% more), yielded 37% more seedlings. Planting 37% less seed would cost $640.15/ha, a 21% decrease in cost. However, it is important to note that increasing or decreasing the seeding rate will not necessarily result in a proportional increase or decrease in seedling establishment (Williams and Smith 2007). Despite its effectiveness, further research on how to use this treatment most effectively would be beneficial before applying this method on a large scale. If this treatment were to be optimized for economic benefits, a logical next step

would be to design an experiment using supplemental seed on prairies with different seeding rates to see which combination results in the highest seedling establishment for the lowest cost.

Management seems to be an important factor in plant establishment as well. I observed a strong treatment effect across all sites combined; however, I only observed a significant treatment effect at Linn County when I analyzed each site separately. Lack of a treatment effect at the Benton sites could have also been influenced by the lack of mowing. Without being mowed, Benton County still had a lot of standing cover crop throughout the growing season. As mowing is recommended the first year to increase light availability to seedlings, the lack of this management strategy at the Benton sites could very well have influenced early establishment (Williams, Jackson, and Smith 2007). Direct observation supports this, as seedlings in areas where the cover crop was very dense were frequently smaller and light starved. Theoretically, there was an equal amount of cover crop across each site, which means mowing would have the same effect on both the treatment and control plots. However, if the benefit of having cover in spring/early summer is more important to predators than a plentiful seed source, the effect of supplemental seed may have been swamped by the benefit of cover over all of the plots. These results and observations suggest that this treatment works most effectively where there is ideal first year management of the sites. Ideal management includes mowing the sites down to 4-6” once the vegetation has reached 12-18” (Smith et al. 2010). In fact, the supplemental seed treatment was most beneficial at Linn, the

site with the lowest seeding rate and most optimal management (proper mowing, drill seeding, and cover crop establishment). However, it is unknown which specific management action or combination of actions was crucial for the success at this site.

Another difference in management of the sites in this study was planting method. Though the treatment effect was significant on the backslopes, some of the backslopes were drilled and some were hydroseeded. This experiment was not set up to test differences between the methods, but the application of supplemental seed seemed to be an effective strategy to reduce seed predation, regardless of the method.

In addition to management, it is important to consider the non-uniformity of roadside sites for prairie restoration. This study showed that sections of the ditch profile can have different rates of early establishment. In addition, the significant site/section interaction shows that sites can differ from each other in which sections of the ditch profile have the better rate of establishment. Factors influencing this significant interaction could include soil moisture, disturbance level, and density of cover crops. For example, there was standing water and saturated soil in the bottoms of Benton S and Linn, but not in the bottoms of Benton N. Benton N was a more upland site overall, Linn foreslopes were heavily influenced by gravel deposition by snowplows, and Benton S had a much denser cover crop than the other sites in some sections of the ditch profile. These differential establishment rates highlight the highly variable nature of roadsides and the challenge of implementing restorations in this setting. This makes it especially important to consider the nature of a site (drainage, disturbances, steepness, etc.) when

considering it for a restoration project, or as a candidate for a supplemental seed treatment.

Another factor to consider is the best time of year to utilize this treatment. Though applying the treatment at the same time as a fall planting was very successful in this study, the seed card method did not reveal this time period as a major predation window. Planting in the spring and applying this treatment could have similar or different effects as even within a system, predation is temporally highly variable (Janzen 1971). Using more seed card studies at different intervals after the planting, and testing this treatment on spring plantings may return more information on when predators are active in roadsides and thus, an even better time to apply the supplemental seed.

At the current level of knowledge, this treatment is a promising strategy to improve prairie restorations, in and beyond roadsides. Future predator satiation studies should focus on how to use supplemental seed most effectively, explore use of the treatment in sites with unique drainage issues and disturbances, and study differential establishment on different sections of the right-of-way profile. Through the use of seed cards at different times of year, future studies can learn more about peak predation times and target the best window for supplemental seed application. Though this study was in roadside rights-of-way, this treatment should be explored in other prairie restoration settings. The same types of studies on timing, location of application, drainage issues, and disturbances would still be necessary in order to maximize the effect of a supplemental seed application. A similar treatment could be used in other

ecosystems as well, but it would be necessary to conduct studies on the types and timing of predators present. As with any restoration, the success of a project will be determined by careful planning and consideration of the site's characteristics and ideal management. Using a supplemental source of seed in order to satiate predators could reduce seed predation on native seed, resulting in more established seedlings, and eventually a higher quality restoration.

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APPENDIX

EARLY SEEDLING ESTABLISHMENT DATA

Table A1: Linear model ANOVA for basal area of early seedlings established at all sites.

Source	Df	SS	MS	F	p
Site	2	30.755	15.378	8.9397	0.0003529 ***
Treatment	1	9.725	9.725	5.6535	0.0201964 *
Section	2	27.214	13.607	7.9104	0.0008071 ***
BA cover crop	1	39.754	39.754	23.1110	9.631e-06 ***
Site x Treatment	2	1.524	0.762	0.4431	0.6438541
Treatment x Section	2	0.129	0.064	0.0375	0.9632216
Site x Section	4	33.283	8.321	4.8372	0.0016928 **
Residuals	69	600322	8576		

Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table A2: Linear model ANOVA for early forb seedlings established at all sites.

Source	Df	SS	MS	F	p
Site	2	8.464	4.2322	3.0904	0.0517429
Treatment	1	16.793	16.7933	5.6535	0.0008091 ***
Section	2	25.783	12.8913	9.4134	0.0002396 ***
Site x Treatment	2	1.982	0.9908	0.7235	0.4886374
Treatment x Section	2	4.629	2.31	1.6901	0.1949349
Site x Section	4	15.376	3.8439	2.8068	0.0319960
Residuals	70	95.863	1.3695		

Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table A3: Linear model ANOVA for early grass seedlings established at all sites.

Source	Df	SS	MS	F	p
Site	2	0.8654	0.43271	1.4896	0.232511
Treatment	1	3.0324	3.03239	10.4393	0.001881 **
Section	2	4.0467	2.02333	6.9655	0.001742**
Site x Treatment	1	1.1126	0.55630	1.9151	0.154965
Treatment x Section	2	0.4463	0.22313	0.7681	0.467744
Site x Section	4	0.4395	0.10989	0.3783	0.823402
Residuals	70	20.3335	0.29048		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

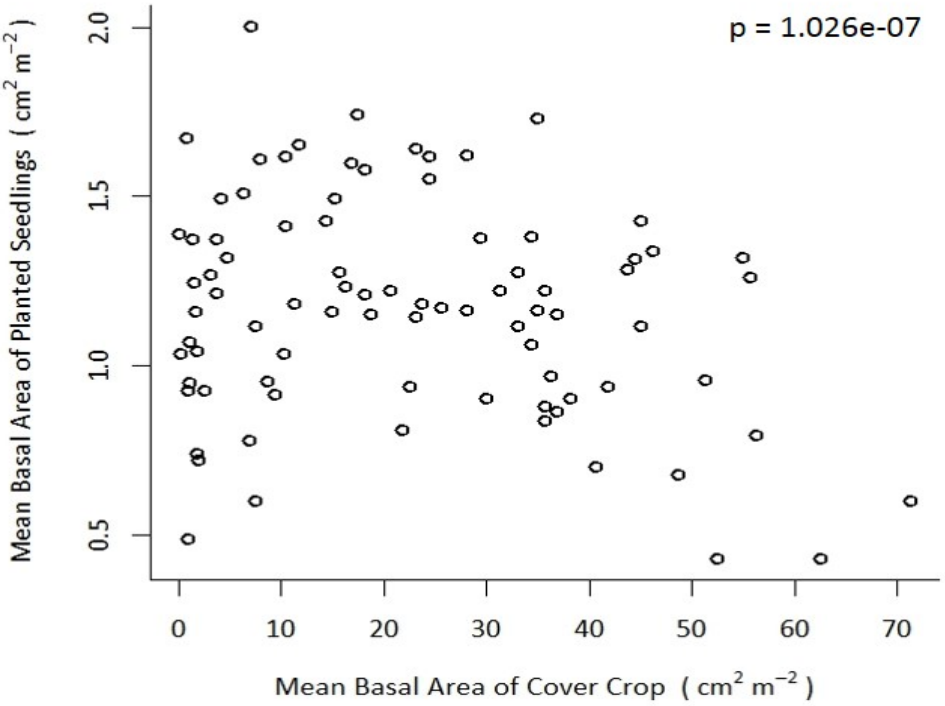

Figure A1: Correlation between Basal Area Cover Crop and Basal Area of Seedlings